A Brief Look into Spike Sorting Methods

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ABSTRACT

Spike sorting is a class of techniques used in the analysis of electrophysiological data. Studying the dynamics of neural activity via electrical recording relies on the ability to detect and sort neural spikes recorded from a number of neurons by the same electrode. This article reviews methods for detecting and classifying action potentials, a problem commonly referred to as spike sorting.

1. Introduction

One of the major questions contemporary neuroscience seeks to elucidate concerns the mechanisms used by dedicated parts of brains (our brain, vertebrate brains, insect brains) to perform specific tasks. Our brains are buzzing with electrical activity created by sodium and potassium ions moving in and out of neurons through specialized pores. Classical methods for exploring the mechanisms of brain function involve recording the electrical activity of single nerve cells. Neurophysiologists often record the currents flowing across cell membranes using an insulated wire probe whose ‘listen’ to a few neurons close-by the electrode tip that fire action potentials or ‘spikes’ (Fig.1).Any such probe will record currents passing across all sorts of physiological membranes close to its tip, but we can approximate this as a small number of close, high-amplitude sources imposed on a background of synaptic, somatic and axonal currents from more distant sources. Each neuron has spikes of a characteristic shape, which is mainly determined by the morphology of their dendritic trees and the distance and orientation relative to the recording electrode (Lewicki, 1998).When a recording electrode measures action potentials from multiple cells, these contributions must be disentangled from the background noise and from each other before the activity of individual neurons can be analyzed. This procedure of estimating one or more single cell point processes from a noisy time series is known as spike sorting. Spike sorting, then, is the process of identifying the waveforms associated with action potentials in the foreground cell(s) and thereby detecting firing events. As a matter of fact, Spike sorting is the grouping of spikes into clusters based on the similarity of their shapes. Given that, in principle, each neuron tends to fire spikes of a particular shape, the resulting clusters correspond to the activity of different putative neurons. The end result of spike sorting is the determination of which spike corresponds to which of these neurons.

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Spike sorting _i.e. the classification of which spike corresponds to which neuron_ is a very challenging problem. Then, before tackling technical issues, it is important to discuss why we need to do such a job, rather than just detecting the spikes for each channel without caring from which neuron they come. It is already well established that complex brain processes are reflected by the activity of large neural populations and that the study of single-cells in isolation gives only a very limited view of the whole picture (Brown, Kass, & Mitra, 2004; Buzsáki, 2004)Therefore, progress in Neuroscience relies to a large extent on the ability to record simultaneously from large populations of cells. The implementation of optimal spike sorting algorithms is a critical step forwards in this direction, since it can allow the analysis of the activity of a few close-by neurons from each recording electrode. This opens a whole spectrum of new possibilities. For example, it is possible to study connectivity patterns of close-by neurons(Buzsáki, 2004; Harris, 2005; Harris, Henze, Csicsvari, Hirase, & Buzsáki, 2000), or to study the topographical organization of a given area and discriminate the responses of nearby units(Lewicki, 1998; Quian Quiroga, 2009; Quiroga, 2007). It is also possible to have access to the activity of sparsely firing neurons, whose responses may be completely masked by other neurons with high firing rates, if not properly sorted. Separating sparsely firing neurons from a background of large multi-unit activity is not an easy job, but this type of neurons can show striking responses (Hahnloser, Douglas, & Hepp, 2002; Perez-Orive, et al., 2002; Wood, Black, Vargas-Irwin, Fellows, & Donoghue, 2004).

General Framework: Why is spike sorting important?

The short answer to this is that it is vital for extra-cellular recording from multiple cells. Obviously, sorting spikes from a single electrode can provide signals from more than one cell. But automatic recognition is also essential for any multiple electrodes recording as achieving and maintaining isolation on multiple probes simultaneously can be highly impractical. The action potentials originating from different recorded neurons have distinct shapes, and based on these differences one can select (or discriminate) certain neurons among others.

2. Spike Sorting Steps

2.1. Data Acquisition

The first step in any spike sorting algorithm involves the acquisition of extracellular data in a form amenable to the detection of neuronal spikes. For many neurons, the most prominent feature of the spike shape is its amplitude, or the height of the spike. One of the simplest ways to measure the activity of a neuron is with a voltage threshold trigger. The experimenter positions the recording electrode so that the spikes from the neuron of interest are maximally separated from the background activity (Quiroga, 2009) . Window discriminators can be implemented on-line, but have the main disadvantage that they require a manual setting of the windows by the user, which may need readjustment during the experiment. In this regard, manual procedures are of limited utility because shape parameters designed for human inspection are inefficient at representing complex waveforms. Moreover the labor-intensive process scales poorly to experiments performed with large numbers of electrodes, and a subjective approach makes it difficult to design reproducible and reportable quality metrics. For these reasons, an algorithmic approach is desirable, and in fact, computational solutions with limited human monitoring have been shown to generally outperform manual sorting (Harris, 2005).

2.2. Filtering

The word "filtering" refers to an attempt to extract the important part of some data while eliminating random contributions called "noise" or other unwanted features which obscure the ones that matter (Quian Quiroga, 2009). The first step when processing continuously recorded data is to apply a band pass filters in order to avoid low frequency activity and visualize the spikes (Quiroga, 2007). This step is usually overlooked in the literature, but its implementation can dramatically change the spike shapes. In the example of Fig. 3, the continuous data was filtered with a band pass filter between 300 and 3000Hz. Frequencies below 300Hz are filtered to delete the slow components of the raw data. The upper cutoff frequency of the filter is to diminish the noisy appearance of the spike shapes. As it is usually the case with filtering, a compromise has to be taken. On the one hand, one would like to have a narrow filter band to better visualize the spikes, but on the other hand, if the band is too narrow, the filter may hinder different features of the spike shapes (Quian Quiroga, 2009; Quiroga, 2007).

2.3. Spike Detection

From the filtered data, spikes are usually detected using an amplitude threshold. An adequate threshold can be set manually, as done in most systems with on-line spike detection.

Although being simple and fast, due to some difficulties, using this method require special vigilance. Moving threshold during recording considered as a next problem. The threshold level determines the trade-off between missed spikes (false negatives) and the number of background events that cross threshold (false positives). In fact increasing the threshold level reduces the number of spikes that are misclassified (Type II error), but at the expense of many missed spikes. On the other hand, getting false positives due to noise crossing a low threshold (Type I error) (Quiroga, 2009; 2007). Another problem in spike detection is misclassification error due to overlaps. In addition to the background noise, the spike height can vary greatly if there are other neurons in the local region that generate action potentials of significant size. If the peak of the desired unit and the dip of a background unit line up, a spike will be missed. (Quian Quiroga, 2009; Quiroga, 2007). However, an automatic threshold is preferable, especially when processing large number of channels. Once spikes are detected, they have to be stored for clustering (Quiroga, 2007).

There are two issues concerning spike storage that need a brief description. The first one is how many data points to store. This of course depends on the sampling frequency and ideally one would like to store the whole spike shape; i.e. about 2 ms of data. With a sampling frequency of 30 KHz, this corresponds to 60 data points. Some methods for feature extraction, such as wavelets (using a multi-resolution decomposition implementation), require that the number of data points is a power of 2 (Nenadic & Burdick, 2005). In this case, with 30KHz sampling, 64 data points would be optimal. The second issue has to do with the alignment of the spike shapes. Spikes can be aligned to their maximum. But due to insufficient sampling the maximum can be at different points of the spike shape. To avoid such misalignments, which could lead to over clustering, the spike shapes can be oversampled using interpolated waveforms, for example, using cubic splines. Then, the interpolated shapes can be aligned and later decimated to the original sampling rate (Quiroga, 2007).

For recordings with good signal to noise ratio (SNR) this is usually achieved by a simple thresholding (Kim & Kim, 2000). When the SNR is not good enough, different spike-detection algorithms can be applied, e.g. use of nonlinear energy operator (Kim & Kim, 2000) continues wavelet transform (Nenadic & Burdick, 2005).

2.4. Feature Extraction

Transforming the input data into the set of features is called feature extraction which involves simplifying the amount of resources required to describe a large set of data accurately. As a third step, it measure features of shapes, such as spike height and width, peak-to-peak amplitude, energy (the square of the signal), mean or variance. In general, the more features we have, the better we will be able to distinguish different spike shapes. The result of this step is an M×K-matrix, where K is the number of detected spikes and M is the number of extracted feature. Principal component analysis (PCA), wavelet decomposition or some other techniques are commonly used to reduce the dimensionality of the M×K-matrix by extracting the most important features of the detected spikes. The result is a new matrix of reduced dimension, L×K, where L < M is the number of extracted features per spike.(Tiganj & Mboup, 2011).

Ideally, one wants to extract those features that best separate the different clusters of spikes and get rid of all the dimensions dominated by noise.This step saves computational time and it is mandatory for some clustering algorithms that cannot handle too many inputs in a reasonable time (Quiroga, 2007). Although eliminating inputs dominated by noise can certainly improve clustering outcomes, but the major challenge is still remain, which two features are the best among extracted features?

A first choice would be to take basic characteristics of the spikes, such as their peak (or peak to peak) amplitude, their width and energy (the square of the signal). However, it has been shown that such features are not optimal for differentiating spike shapes in general (Quiroga, 2007; Sakowitz, Quian Quiroga, Schürmann,

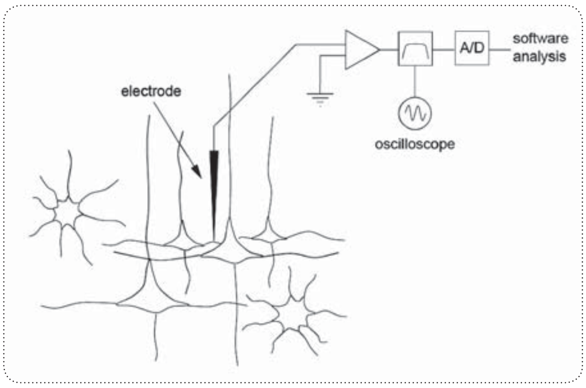

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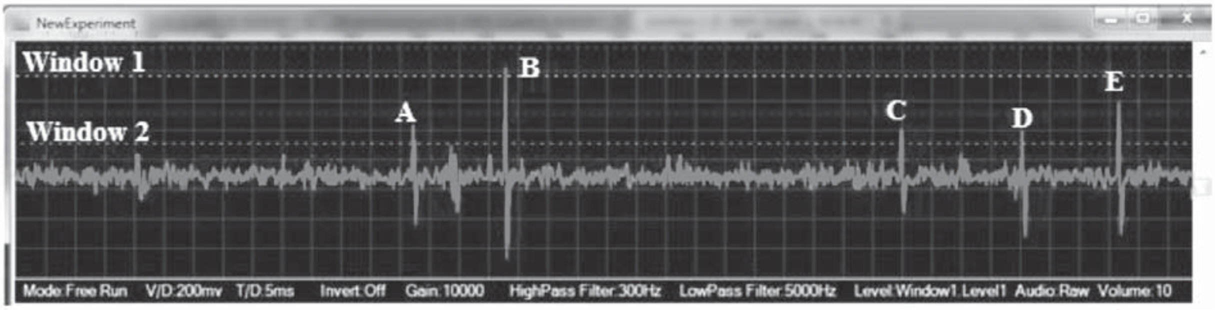
Figure 1. The basic set-up for measuring and analyzing extracellular neural signals.

& Basar, 2005; Sambeth, Maes, Quiroga, & Coenen, 2004). By far, the most used method for feature extraction is to take the first 2 or 3 principal components (Abeles & Goldstein Jr, 1977; Glaser & Marks, 1968), usually containing more than 80% of the energy of the signal (Quiroga, 2007). However, principal component analysis (PCA) selects the directions of maximum variance of the data, which are not necessarily the directions of best separation. In other words, it may well be that the information for separating the clusters is represented in one (or a combination) of principal components with low eigenvalues, which are usually disregarded (Quiroga, 2007).

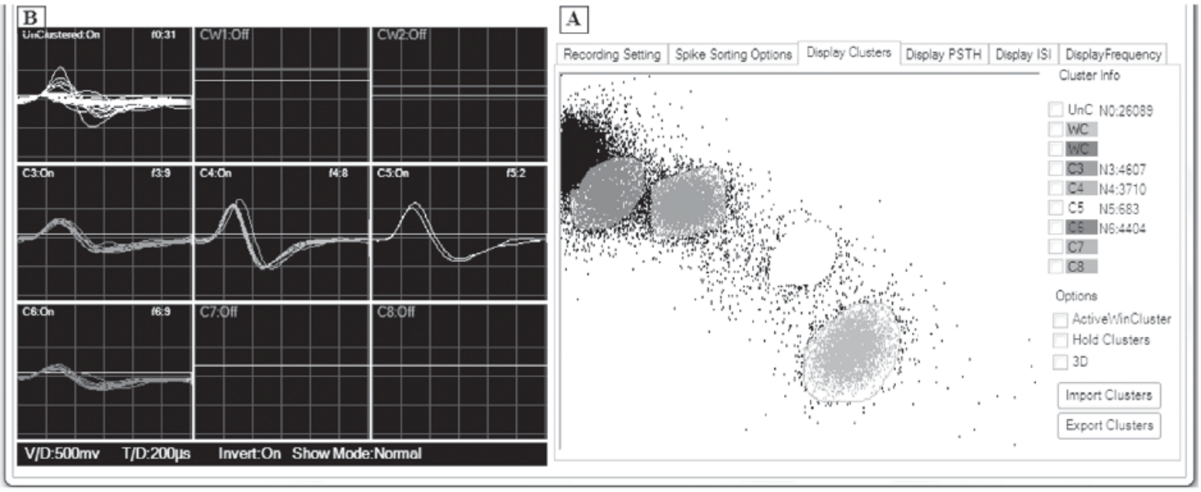
3. Clustering

Cluster analysis or clustering is the task of assigning a set of objects into groups (called clusters) so that the objects in the same cluster are more similar (in some sense or another) to each other than to those in other clusters. The fourth and final step of spike sorting is to group spikes with similar features into clusters, corresponding to the different neurons. A common method is a technique called cluster cutting. In this approach, the user defines a boundary for a particular set of features. If a data point falls within the boundary, it is classified as belonging to that cluster; if it falls outside the boundary, it is discarded. Figure 3(a) shows an example of boundaries placed around the primary clusters. In off-line analysis the cluster boundaries are determined after the data have been collected by looking at all (or a sample from) the data



Mode:Free Run V/D:200mv T/D:5ms Invert:Off Gain:10000 HighPass Filter:300Hz LowPass Filter:5000Hz Level:Window1.Level1 Audio:Raw Volume:10

Figure 2. The extracellular waveform shows several different action potentials generated by an unknown number of neurons. The data were recorded from Rat's locus coeruleus witha Tungsten electrode by Electromodule and Neurocomet (sciencebeam instiitute, Tehran, Iran). In this case, by amplitude discrimination method, neurons C and D are recorded as a same neuron by window 2.



V/D:500mv T/D:200µs Invert:On Show Mode:Normal

Figure 3. (A) Shape separation of the spikes by cluster analysis in the space of waveforms (Neurocomet, Sciencebeam institute, Tehran Iran) Clusters are shown with colors. The axes of the scatter plot are positive and negative area under curve. (B) The separated waveforms resulting from clustering the raw spike waveforms.

over the collection period. This allows the experiment to verify that the spike shapes were stable for the duration of the collection period (Lewicki, 1998). However, besides being a very time-consuming task, manual clustering introduces errors due to the limited dimensionality of the cluster cutting space and due to human biases(Harris, et al., 2000). In fact, in many cases clusters overlap and the manual setting of a boundary is very subjective.

4. Discussion

We have reviewed here the process of spike sorting. In addition to so called methods, there are some other methods for spike sorting including template matching, Bayesian methods and so on. Each method has some advantages and disadvantages itself. Accordingly, template-based methods can fail for neurons that burst and can becomeincreasingly inaccurate if there is electrode drift or Bayesian methods are much more accurate for spike shapes that are similar. On the other hand, despite the simplicity ofamplitude discrimination, this technique can be time consuming and biased. Not onlycan neurophysiologists waste hours searching for well isolated cells, but in the end thissearch is biased towards cells that produce the largest action potentials which may not berepresentative of the entire population. Hence according to pros and cons of each method, a more practical question might be: what is the simplest method that satisfiesexperimental demands? For many researchers this is still a single electrode with threshold detection despite mentioned difficulties.

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